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# Response to "Comment on 'Evaluation of a Gene–Environment Interaction of *PON1* and Low-Level Nerve Agent Exposure with Gulf War Illness: A Prevalence Case–Control Study Drawn from the U.S. Military Health Survey's National Population Sample'"

*Marc G. Weisskopf*[1,2]

[1]Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
[2]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA

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Curtis points to important data about the *paraoxonase-1* (*PON1*) gene[1] that highlight some of the key features of, and a key assumption necessary for, all the causal inference benefits outlined in the invited perspective[2] on the paper by Haley et al.[3] on gene–environment (G × E) interaction between *PON1* and nerve agent exposure in relation to Gulf War illness (GWI) that I coauthored.

As Curtis points out,[1] frequency of the *PON1* R allele differs by ancestry. Therefore, if cases and controls differ in ancestry, there could be allele and genotype differences even if there is no effect of the gene on GWI. Curtis points to the lack of Hardy-Weinberg equilibrium (HWE) among the cases in the study by Haley et al. However, interpreting Hardy–Weinberg equilibrium (HWE) in cases is tricky: Although departure from HWE is an expected consequence of ancestral heterogeneity, it would also occur among the cases if the gene contributed to GWI. Haley et al. found controls to be in HWE.[3] Nonetheless, case–control differences in allele frequency due to ancestry differences raise concern of bias because the adjustment Haley et al. used for race/ethnicity was minimal.[3]

Such case–control differences indeed may well have happened, but importantly, as Curtis points out,[1] the resulting bias would be on the main effect of the gene, not the G × E interaction. (In our invited perspective,[2] we outline the same scenario for recall bias of the environmental exposure.) The other question he poses, though—whether there could also be differences in nerve agent alarm exposure by ancestry—raises the possibility of G × E dependence, a violation of a key assumption necessary for the causal inference benefits of G × E interaction studies described in our perspective.

However, rather than just speculate about this possibility, Haley et al. assessed G × E dependence directly[3]—that is, genotype predicting exposure—in the controls (not among the cases because they would show G × E dependence if a true G × E interaction existed[4]). The authors reported an odds ratio of 1.18 (95% confidence interval: 0.81, 1.73) for the G × E dependence. Thus, regardless of the distribution of ancestry or whether ancestry (only an indirect proxy of genetics) predicts nerve agent alarm exposure, in these data the genotype does not predict exposure. This suggests G × E independence and that the observed G × E interaction is, if anything, biased to the null.[4] Further, even if we ignore the significance and assume there is a dependence at the level of an odds ratio of 1.18, it can be shown mathematically that this level of dependence places a maximal bound on bias of the G × E interaction. This bound is far lower than the G × E interaction found by Haley et al.,[3] further arguing that a true G × E interaction exists.

The important points about the *PON1* gene that Curtis raises[1] further demonstrate the great advantages of G × E interaction studies from a causal inference perspective. Although the allele differences by ancestry are a potential source of bias for the main effect of the genotype, the study data actually suggest that they do not bias the G × E interaction.

## References

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Address correspondence to Marc G. Weisskopf. Email: mweissko@hsph.harvard.edu

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